Human Research Program Flight Analogs Project Information Package

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Flight Analogs Project
Human Research Program

Please use this document as a guide to the standard conditions and measures for the purpose of preparing research protocols. Questions related to this document can be directed to:

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The NASA Flight Analogs Project (FAP) provides a forum for investigating the effects of microgravity on the physiology of the whole body. The information provided in this document describes the standard conditions, standard measures, and services provided by the Flight Analogs Project. The standard platform for bed rest studies is 60 days of six degrees head down tilt (HDT) bed rest. Durations of 30-days and 90-days can also be accommodated if warranted by study requirements. NASA bed rest studies are performed at the Flight Analogs Research Unit (FARU) as part of the Institute for Translational Sciences-Clinical Research Center (ITS – CRC) located at the University of Texas Medical Branch in Galveston, TX. The standardization of studies described in this document are integrated with investigator protocols on a non-interference basis.

1.0 NASA FLIGHT ANALOGS PROJECT

- Provides a set of standardized bed rest study conditions to insure consistency across all studies
- Collects a set of Standard Measures for every bed rest subject
- Maximizes resources by combining individual investigations into integrated studies

2.0 INVESTIGATOR RESPONSIBILITIES

- Meet with Flight Analogs Project team and investigators of other studies to strategize and develop integrated protocols
- Comply, as much as possible, with the Standard Conditions of Bed Rest
- Accommodate, as much as possible, the collection of Standard Measures of bed rest
- Provide for on-site study support at the FARU in Galveston, TX. Collaboration with UTMB investigators may be helpful to meet this need.
- Budget for costs associated with on-site support
- Carry out investigator science according to protocols
- Participate in periodic data debriefs
- Provide complete experimental data sets within 2 years after study completion for inclusion into the Flight Analogs Project database
- Provide manuscript within 2 years of study completion for inclusion into final project report

3.0 INVESTIGATOR PREPARATIONS FOR HUMAN SUBJECTS BOARDS

- Work with the FAP Project Scientist to determine needed approvals from the investigator's home institution.
- Prepare individual protocol submissions to the NASA Committee for the Protection of Human Subjects (CPHS).
- Prepare protocol information for integration into a study complement for submission to the UTMB Institutional Review Board (IRB)

4.0 INVESTIGATOR RESOURCE/FISCAL RESPONSIBILITIES

- The investigator will provide resources for their experiment unique requirements

- The investigator will have responsibility for the costs of any investigator protocol specific screening requirements, equipment, imaging studies, research pharmacy utilization and other investigation specific requirements.
- The investigator is responsible for costs associated with personnel for conduct of their own study, travel, and equipment shipping
- The investigator is responsible for test subject travel costs for follow up testing beyond the standard schedule

5.0 SERVICES PROVIDED BY THE FLIGHT ANALOGS PROJECT TEAM

- Coordinate investigator meetings
- Coordinate preparation and submissions to the human subjects boards.
- Recruit and perform standard FAP subject screening, reimbursement, and transport
- Provide subject consent briefings
- Provide test subject monitors
- Provide attending physicians and medical monitors
- Develop and manage schedules and the associated logistics to implement integrated studies
- Design and implement Case Report Forms (CRF)
- Provide data management support for the collection, transfer, and storage of study data sets
- Establish and maintain a bed rest web site to disseminate information.
- Coordinate logistics for shipment of investigator equipment.
- Coordinate storage requirements for investigator equipment.
- Provide transport of subjects at 6 degrees head-down for testing at remote locations when needed
- Provide test subject and medical staff orientations
- Conduct integrated Test Readiness Reviews, safety walk-throughs and operations check-out prior to study start
- Provide on-site coordinators to support daily testing activities
- Provide a daily operational status report
- Coordinate post study subject follow up testing
- Distribute Standard Measures data to investigators.

6.0 BED REST STANDARDIZED CONDITIONS

- Duration: 60 days
- Bed Position: 6 degrees head down tilt, continuous for the duration of the study
- Room Temperature: 72° F. (+/- 2 degrees)
- Humidity: 70% (+/- 5%)
- Light/Dark Cycle: Lights on 0600, lights out 2200, 7 days per week, no napping is permitted
- Daily Measurements:

  - Blood Pressure, Heart Rate, Respiratory Rate, Body Temperature
  - Body Weight
  - Fluid Intake and Output
- Monitoring: By Subject Monitors in person or via in-room camera 24 hours a day
- Stretching Regimen: Twice daily
- Physiotherapy: Every other day during bed rest and every day for the first seven days post bed rest
- Psychological support is provided once weekly during the study and available as needed at all times

7.0 BED REST STANDARDIZED DIET

- Metabolically controlled diet based on the NASA space flight nutritional requirements
- Carbohydrate:Fat:Protein ratio – 55:30:15
- Minimal fluid intake of 28.5 ml/kg body wt (2000 ml/70 kg subject); additional water may be consumed
- No caffeine, cocoa, chocolate, tea or herbal beverages
- All food must be consumed
- Caloric intake adjusted to maintain weight within 3% of day 3 of head down tilt
- Iron supplementation is provided for all female subjects
- Iron supplementation is provided for male subjects with a low ferritin (less than 35 ng/ml) at study entry
- Vitamin D supplementation (800 IU/day) is provided throughout bedrest
- Subjects with low vitamin D levels (less than 50 nmoles/L) at study entry will be supplemented during the pre-bed rest phase

Nutrient	Bed Rest Recommendation	Flight Recommendation
Energy, kcal	Maintain BW	WHO (moderate activity)
Protein, g	12-15% of total energy	12-15% of total energy
% kcal from fat	30-35% of total energy	30-35% of total energy
% kcal from protein	12-15% of total energy	12-15% of total energy
Vitamin A, μg RE	1000 μg RE	1000 μg RE
Vitamin D, μg RE	10 μg RE (400 IU)	10 μg RE (400 IU)
Vitamin E, mg α-TE	20 mg α-TE	20 mg α-TE
Vitamin K, μg	80 μg (M), 65μg (F)	80 μg (M) 65μg (F)
Vitamin C, mg	100 mg	100 mg
Thiamin, mg	1.5 mg	1.5 mg
Riboflavin, mg	2.0 mg	2.0 mg
Niacin, mg	20 mg	20 mg
Pantothenic acid, mg	5.0 mg	5.0 mg
Vitamin B6, mg	2.0 mg	2.0 mg
Folate, μg	400 μg	400 μg
Vitamin B12, μg	2.0 μg	2.0 μg
Calcium, mg	1000 – 1200 mg	1000 – 1200 mg
Phosphorus, mg	1000 – 1200 mg	1000 – 1200 mg
Magnesium, mg	350 mg (M), 280 mg (F)	350 mg (M) 280 mg (F)
Iron, mg	10 mg (M), 18 mg (F)	10 mg
Zinc, mg	15 mg	15 mg
Copper, mg	1.5-3.0 mg	1.5-3.0 mg
Selenium, μg	70 μg	70 μg
Sodium, mg	<3500 mg	<3500 mg
Potassium, mg	3500 mg	3500 mg
Fiber, g	10-25 g	10-25 g
Manganese, mg	2.0-5.0 mg	2.0-5.0 mg

Figure 1: Bed Rest Nutrition Intake Recommendations

8.0 BED REST STANDARD MEASURES

BACKROUND

An overview of the standard measures to be performed during all flight analog/bed rest studies is presented here. Standard measures provide a characterization of the physiologic responses to bed rest in humans across disciplines. These protocols are performed at the Institute for Translational Sciences-Clinical Research Center (ITS –CRC) and are integrated with science investigation requirements on a non-interfering basis. The standard measures can be utilized to describe gender differences in the physiological responses to bed rest, provide a basis for comparisons of bed rest results with results from spaceflight investigations, and provide ancillary data to individual investigators. A schedule of the standard measures is located after the descriptions.

BONE MINERAL DENSITY (DXA)

Dual Energy X-Ray Absorptiometry (DXA) will be used to obtain measures of bone mineral density. A Hologic Discovery Unit whole body densitometer will be used to perform scans, and scan analyses will be performed using the standard analysis software provided by Hologic. Bone mineral density will be determined for the whole body and for regional bone sites at both hips, lumbar spine, calcaneus, and forearm. All six scans are performed in triplicate.

	EDE* (Men)	**EDE* (Pre-menopausal Women)**
DXA (triplicate scans)		
Whole body	0.78 mREM	1.02 mREM
Hip (R and L)	0.24 mREM	2.20 mREM
Lumbar Spine	0.40 mREM	0.40 mREM
Calcaneus	0.01 mREM	0.01 mREM
Forearm	0.01 mREM	0.01 mREM
Total	1.44 mREM	3.64 mREM
QCT		
Hip	17 mREM	30 mREM
Spine	11 mREM	11 mREM
Total	28 mREM	41 mREM

*EDE = effective dose equivalent

Figure 2. Associated Radiation Exposure Per Session

BONE MASS AND GEOMETRY (QCT)

Quantitative Computerized Tomography (QCT) will be used to evaluate bone mass and geometry. Lumbar and hip scans will measure bone density and analyze regional changes in bone. QCT scans will be analyzed to determine volumetric bone mineral density (vBMD), bone mineral content, and bone size.

CLINICAL NUTRITIONAL ASSESSMENT

General blood and urine chemistry, electrolytes, selected markers of hematological, protein, vitamin and mineral status, markers of oxidative damage, and markers of bone metabolism will be assessed. In addition, cellular mineral content (sodium, potassium, chloride, calcium, phosphorous, magnesium) will be will be analyzed from sublingual epithelial cells collected with a wooden spatula by scraping the floor of the mouth.

Serum Measurements for Nutrition Standard Measure

Chemistry

Sodium, Potassium, Chloride, Creatinine, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Cholesterol, LDL Cholesterol, HDL Cholesterol, Triglyceride, hs-CRP, IL-1 beta, Total Lipids, TNF-alpha

Portable Clinical Blood Analyzer

Hemoglobin, Hematocrit, Ph, Ionized Calcium, Potassium, Sodium, Glucose

Mineral Status

Zinc, Selenium, Iodine, Copper, Ceruloplasmin, Phosphorus, Magnesium

Calcium and Bone Metabolism Markers

25-Hydroxyvitamin D, 1, 25-Dihydroxyvitamin D, Intact Parathyroid Hormone (PTH), Osteocalcin, Alkaline Phosphatase, Bone Specific Alkaline Phosphatase (BSAP), Serum Calcium, Osteoprotegerin (OPG), Osteoprotegerin ligand (receptor activator of nuclear factor-kB ligand or RANKL), Insulin-like Growth Factor, Leptin

Hematologic and Iron Status Indicators

Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Transferrin Receptors, Transferrin, Ferritin, Ferritin Iron, Ferritin Iron % Saturation, Iron, Fibrinogen

Protein Status

Retinol Binding Protein, Transthyretin, Total Protein, Albumin, Alpha 1 globulin, Alpha 2 globulin, Beta globulin, Gamma globulin

Hormones

Testosterone, Free Testosterone, Estradiol, Dehydroepiandrosterone (DHEA), Dehydroepiandrosterone Sulfate (DHEA-S), Cortisol

Water Soluble Vitamin Status

Erythrocyte Transketolase Stimulation, Erythrocyte Glutathione Reductase Activity, Erythrocyte nicotinamide adenosine dinucleotide and nicotinamide adenosine dinucleotide phosphate (NAD/NADP), Erythrocyte Transaminase Activity, Folate, RBC and Serum, Homocysteine, Vitamin C, Pyridoxal 5-phosphate (PLP)

Fat Soluble Vitamin Status

Retinol, Retinyl palmitate, β-carotene, Serum Phylloquinone, α-tocopherol, γ-tocopherol, Tocopherol : lipid ratio, Vitamin D Binding Protein, Plasma Heme, Undercarbolxylated Osteocalcin

Antioxidants and Markers of Oxidative Damage

Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPX), Malondialdehyde (MDA), Total Lipid Peroxides Glutathione Protein Carbonyls, Reduced and Oxidized Glutathione

Urinary Measurements for Nutrition Standard Measure

General

Total volume, pH, Creatinine, Chloride, Cortisol

Bone Metabolism Markers
N-telopeptide (NTX), Pyridinoline (PYD), Deoxypyridinoline (DPD), γ-carboxy glutamic acid, C-telopeptide (CTX), Helical Peptide (HP)

Minerals
Calcium, Phosphorus, Magnesium, Copper, Selenium, Zinc, Iodine

Water Sol. Vitamins
N-methyl nicotinamide, 2-pyridone, 4-pyroidoxic acid

Protein Status
3-methyl histidine, Nitrogen

Antioxidants
8-OH deoxyguanosine, Prostaglandin F2 α (PG F2 α)

Renal Stone Risk
Sodium, Potassium, Uric Acid, Citrate, Oxalate, Sulfate, Supersaturation of Calcium Oxalate, Brushite, Struvite, Urate

CLINICAL LABORATORY ASSESSMENT

Additional blood and urine studies will be performed to monitor subject health status and provide additional data.

Serum Measurements for Clinical Laboratory Standard Measure

Chemistry Profile
Carbon Dioxide, Chloride, Creatinine, Glucose, Potassium, Sodium, Blood Urea Nitrogen, Glomerular Filtration Rate, Phosphorous, Magnesium, Bilirubin, Glutamyltransferase, Alkaline Phosphatase, Lactate Dehydrogenase, Creatine Kinase, Uric Acid, C Reactive Protein (hs CRP) Aspartate Transaminase, Alanine Transaminase, albumin, total protein, calcium.

CBC/differential/platelets
White Blood Count and differential, Red Blood Count, Hemoglobin, Hematocrit, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Relative (Red Cell) Distributive Width (RDW), Platelet Count, Reticulocyte Count

Iron Profile
Iron, Total Iron Binding Capacity (TIBC), Transferrin, Transferrin Saturation, Ferritin

Ionized Calcium Profile
Serum Ionized Calcium, pH-Serum, Ionized Calcium at pH 7.40

Hormones
Thyroxine (Free T4), Thyroid Stimulating Hormone (hTSH III)

Urinary Measurements for Clinical Laboratory Standard Measure

Urinanalysis

Specific Gravity, pH, Color, Appearance, Protein, Glucose, Bilirubin, Urobilinogen, Ketone, Nitrite, Blood, Leukocyte Esterase,

Other

Creatinine

ISOKINETIC MUSCLE FUNCTION TESTING

Isokinetic muscle strength and endurance tests will be conducted prior to and following bed rest. Muscle performance testing will be administered using a standard clinical isokinetic dynamometer (Biodex) on selected muscle groups. A standard protocol for warm-up prior to testing will be followed for each muscle group. Testing will be performed on the right limb. The protocol will be performed as described below:

Warm-up	5 minutes at 50 Watts on upright cycle ergometer
Knee Flexion/ Extension (Seated):	• Position Subject • Set Range of Motion (20-95 degrees) • 5 submaximal concentric repetitions at 60 deg/sec: extension and flexion • 5 concentric maximal reps at 60 deg/sec: extension • 5 concentric maximal reps at 60 deg/sec: flexion • 2-3 submaximal concentric repetitions at 180 deg/sec: extension and flexion • Endurance test: 20+1 reps. at 180 deg/sec (extension and flexion)
Ankle Platar/ Dorsiflexion (Prone):	• Position Subject • Set Range of Motion (minimally -15 to +30 degrees) • 5 submaximal concentric repetitions at 30 deg/sec: extension and flexion • 5 concentric maximal reps at 30 deg/sec: plantar flexion • 5 concentric maximal reps at 30 deg/sec: dorsiflexion • 2-3 submaximal eccentric repetitions at 30 deg/sec: extension and flexion • 5 eccentric maximal reps at 30 deg/sec: plantar flexion • 5 eccentric maximal reps at 30 deg/sec: dorsiflexion
Trunk Extension/ Flexion (Standing)	• Position Subject • Set Range of Motion (0-90 degrees) • 5 submaximal concentric repetitions at 30 deg/sec: extension and flexion • 5 concentric maximal reps at 60 deg/sec: extension • 5 concentric maximal reps at 60 deg/sec: flexion

Figure 3. Isokinetic Protocol

AEROBIC CAPACITY – CYCLE ERGOMETTRY

To assess cardiovascular functional performance, maximum aerobic capacity will be measured in each subject using a graded cycle exercise test. Subjects will pedal a cycle ergometer at increasing work loads (50-350 Watts) while heart rate and rhythm, blood pressure, and oxygen uptake are monitored. Exercise tests will be conducted twice prior to bed rest and twice following bed rest. These tests will be performed on a cycle ergometer (LODE^{TM} Excalibur

Sport) with the subject in the upright (seated) position. The testing protocol and equipment will be the same as the standard NASA Medical Operations test used to determine peak cycle heart rate (HR) and oxygen consumption. Details of the test protocols are shown below.

Protocol A		Protocol B	
Work Rate (Watts)	**Time (min)**	**Work Rate (Watts)**	**Time (min)**
50	3	50	3
100	3	75	3
150	3	100	3
175	1	125	1
200	1	150	1
225	1	175	1
250	1	200	1
+25*	+1*	+25*	+1*

Figure 4. Cycle Ergometer Exercise Protocols[a] for Determination of Peak VO_2.

* Test continues until subject reaches peak effort. Peak effort is verified as: (1) Plateau in VO_2 despite a work rate increase, or (2) Attainment of HR > 90% age-predicted maximum accompanied by a respiratory exchange ratio (VCO_2/ VO_2) of greater than 1.10.

[a] Protocol A is used for subjects weighing > 65 kg. Some discretion is used for the assignment of protocols. For example, Protocol A is also appropriate for a 62 kg individual who regularly performs cycle exercise.

FUNCTIONAL FITNESS

The Functional Fitness Test is used by the Astronaut Strength Conditioning and Rehabilitation team (ASCR) to establish baseline muscle strength, endurance, and flexibility before space flight and to monitor their rehabilitation after long duration missions. Data from bed rest subjects will provide a comparison of bed rest to space flight and a tool for monitoring test subject rehabilitation. Free weight muscle strength and endurance tests are functional strength measurements because they are closely associated with activities of daily living (ADL), such as standing up from a chair or bending to lift a box which involves production of force to overcome the inertia of the body mass as well as the inertia of the external object lifted. Additionally, ADL's are multi-joint and multi-muscle activities. Before and after bed rest, subjects will perform two 1-repetition maximum (1-RM) tests on a Cybex™ leg press machine. Subjects will warm up with 5 minutes on a cycle ergometer at 50 to 100 watts. After cycling, the subjects will execute a stretching routine followed by a ramp-up procedure on the leg press: 1x8 (50% projected 1-RM), 1x5 (60% projected 1-RM), 1x3 (70% projected 1-RM), 1x1 (80% projected 1-RM), 1x1 (90% projected 1-RM), and 1x1 (100% of projected 1-RM). Subjects will continue to perform 1 repetition with weight increasing by 3% to 5% until failure. They will be given a 2 to

5-minute rest between sets. The maximum amount lifted will be recorded and documented as the subject's 1-RM. In addition to free weight muscle strength, subjects will perform a 2 minute push up and sliding crunch test to measure local muscle endurance according to the guidelines outlined by the United States Military. They will also perform a pull up test to failure to measure muscle endurance/ strength, and a sit and reach test to measure lower back and hamstring flexibility.

COMPUTERIZED DYNAMIC POSTUROGRAPHY

Sensorimotor balance control function will be tested before and after bed rest using a NeuroCom Equitest Computerized Dynamic Posturography System (Clackamas, OR). During these sessions, the subject stands on a movable, force-sensing, support surface and within the movable visual enclosure of the EquiTest system. Movements of the support surface and/or visual enclosure, under precise computer control are used to modify the sensory conditions and/or to impose unexpected perturbations to standing posture. Tests are performed with eyes open, eyes closed and head movements to challenge senory systems while maintaining upright stance. Subjects wear a harness attached to an overhead system to prevent falls should they lose their balance during testing. The position of the head and other body segments are monitored using an Optotrak motion analysis system (NDI, Ontario, Canada) which, in conjunction with the EquiTest system, is used to determine the type of strategy the subject uses to maintain balance. The subject will be instrumented with three electrocardiogram (ECG) electrodes for every test session and data (ECG/heart rate) will be collected. Testing is conducted twice before bed rest to achieve an accurate baseline and up to five times after bed rest to assess recovery. A third test may be conducted before bed rest as needed to ensure an adequate baseline.

T-REFLEX TEST

Subjects will lie in the prone position on a device developed specifically for left ankle dorsiflexion/plantarflexion. Electromyogram (EMG) electrodes with a high impedance probe will be placed on the tibialis anterior muscle, the medial and lateral gastrocnemius muscles, the medial and lateral soleus muscles, and the gastroc-soleus interface muscle.With the left foot firmly attached to a footplate in a position of –5° degrees of dorsiflexion (selected to preload the tendon stretch), an 80 ft. lb. DC servomotor controlled via position feedback will provide a 10° dorsiflexion about the subject's ankle. Subjects will remain relaxed during the 20 trials, enabling collection of the T-reflex data. EMG will be collected using a Bagnoli-8 EMG amplifier system. Supplementary data (motor torque, velocity, and position) will be collected simultaneously with the EMG and digitized via a 16-bit data acquisition card and sampled at 4000 Hz. EMG data will then be analyzed for both latencies and amplitudes.

CARDIOVASCULAR ASSESSMENT

Neuroendocrine and Cardiovascular Responses to Tilt

Subjects will be placed on the tilt table, and blood drawn for measurement of neuroendocrine parameters. The subject will then be tilted upright at 80 degrees for 30 minutes. Then a final sample will be drawn for a repeat of the supine blood measurements. If the subject cannot tolerate 30 minutes of upright tilt, the tilt table will be placed in the -10° head down position and the blood drawn immediately.

Plasma Volume

The objective of this test is to measure blood (plasma) volume using the carbon monoxide re-breathing technique. The subject will lie supine for 10 minutes. The subject will breathe 100% oxygen on a closed system for 2 minutes. Twenty-eight cc of carbon monoxide will then be injected into the system and rebreathed for 10 minutes, after which a blood sample will be drawn for analysis for hematocrit, total hemoglobin and carboxyhemoglobin. Following the first blood draw, 32 cc of carbon monoxide will be injected into the system and rebreathed for 10 minutes, after which a second blood sample will be drawn. The subject will be continuously breathing on the closed circuit for up to 25 minutes.

Cardiac Function

Echocardiography will be performed on bed-rest subjects utilizing views consistent with standards established by the American Society of Echocardiography. Hemodynamic assessment will be obtained by use of continuous, pulsed wave and color flow Doppler. All four cardiac valves will be evaluated for regurgitation. Velocity measurement of tricuspid regurgitation, if present, provides an accurate measurement of the pressure difference between the right ventricle and right atrium and leads to an estimation of peak pulmonary pressure. The acceleration time of flow through the pulmonary artery can also give an estimation of pulmonic pressure.

To address diastolic function, several traditional echocardiographic measurements and indices (including mitral E and A wave velocities, mitral deceleration time and isovolumic relaxation time) will be employed in addition to more investigational measures.

Many of the same parameters acquired during supine rest are also to be acquired during upright tilt after the subject has been upright for over one minute. Not only will this address the above cardiac parameters under differing loading conditions; but the difference between the supine and upright values can offer an insight into the ability of the heart and cardiovascular system to adapt to a stress following bed rest. The measurements included during tilt include Doppler evaluation of the mitral, tricuspid and aortic valves in addition to tissue Doppler of the lateral annuli of the mitral and tricuspid valves and color M-mode flow propagation velocity. Two and three dimensional imaging of the heart also allows for subsequent volume measurement.

IMMUNE FUNCTION ASSESSMENT

General Immune Status

A general immune assessment will be performed, consisting of white blood cell count and differential, immunophenotype distribution, T cell function, and intracellular cytokine profiles. Regarding immunophenotype, the following peripheral leukocyte populations will be assessed:

leukocyte differential, lymphocyte subsets, T cell subsets, T cell subset memory/naïve ratio, levels of peripheral activated T cells. For T cell function, whole blood cultures will be pulsed with mitogenic stimuli, following which T cell expression of activation markers will be assessed. Intracellular cytokine profiles consist of IFNg and IL-2 expression T cell subsets following PMA+ionomycin stimulation in the presence of monensin to allow intracellular accumulation.

The phenotypic analysis of leukocyte subpopulations will be performed to correlate with the results from the WBC and differential hematology data. The antibody combinations for immunophenotype and functional analysis by flow cytometry are as follows:

LEUKOCYTE SUBPOPULATION DISTRIBUTION

FITC	PE	ECD	PercP	CELL POPULATIONS ASSESSED
CD14	CD19		CD45	WBC Differential/B-cells
CD3	CD56		CD45	Lymphocytes subsets
CD4	CD8		CD3	T cell subsets
CD45RA	CD45RO	CD8	CD3	T cell subsets; memory/naïve T cell subsets
HLA-DR	CD69	CD8	CD3	Early, late activated T cell subsets

CYTOKINE PRODUCTION PROFILES

FITC	PE	ECD	PercP	
IL-2	IFNg	CD8	CD3	T cell cytokine profiles

T CELL FUNCTION (24 hr ctl, A+B, 3/28)

FITC	PE	ECD	PercP	
CD25	CD69	CD8	CD3/4	Culture activated T cell responses.

Figure 5. Antibody Combinations for Immunophenotype and Functional Analysis by Flow Cytometry

Viral-Specific Immunity

For characterization of virus-specific T-cells, flow cytometric assays (tetramer staining and intracellular cytokine staining) for virus EBV-specific T-cells will be performed.

Latent Viral Reactivation

Standard techniques (immunofluorescence assay) will be used on serum/plasma specimens for determining Immunoglobulin (Ig)G/IgM antibodies to Epstein-Barr virus (EBV), viral capsid antigen, early antigen, EBV nuclear antigen and cytomegalovirus (simultaneous detection of immediately early, early, and late antigens). Measurement of an irrelevant antibody for an acute virus infection (i.e., anti-measles virus) will be performed to confirm the specificity of EBV-antibody titer changes. Viral load in blood, saliva, and urine samples will be measured using polymerase chain reaction (PCR) methodology.

Physiological Stress

Plasma, urinary, and salivary cortisol will be determined before, during, and after bed rest.

NASA FLIGHT ANALOGS PROJECT STANDARD MEASURE TESTING SCHEDULE

For all bed rest subjects the study is divided into three phases: a pre-bed rest phase of 13 days for acclimation and baseline data collection, a 60-day bed rest phase, and a post-bed rest recovery phase of 14 days for post-bed rest testing and reconditioning. Each study day is referred to with a conventional naming system. Pre-bed rest days begin at BR-13 and end on BR-1. Days in bed rest begin on BR1. Post-bed rest days begin on BR+0 and subjects are released on BR+13.

Standard Measure	Testing Days Planned			NASA JSC LSRL
	Pre	During	Post	
Bone Densitometry (DXA)	-13		+2	Bone and Mineral
Bone Mass and Geometry (QCT)	-3		+4	Bone and Mineral
Clinical Nutritional Assessment	-10, -3	28	+0, +5	Nutritional Biochemistry
Clinical Laboratory Assessment	-10,	28	+0, +5	Clinical
Cycle Ergometry	-12, -7		+0, +11	Exercise Physiology
Isokinetic Testing	-11, -6		+2, +12	Exercise Physiology
Functional Fitness	-10, -5		+3, +13	*ASCR
Computerized Dynamic Posturography	-10, -4		+0, +1, +2, +4, +8	Neurosciences
T-Reflex	-10, -4, -1	5, 20, 60	+0, +3, +5	Neurosciences
Neuroendocrine and Cardiovascular Response to Tilt	-5		+0, +3	Cardiovascular
Plasma volume	-5	3, 21, 31	+0, +3	Cardiovascular
Cardiac Function	-5	7, 21, 31	+0, +3, +13	Cardiovascular
Immune Function Assessment	-10	28	+0, +5	Immunology/Microbiology

*ASCR-Astronaut Strength Conditioning and Rehabilitation team

Figure 6. NASA Flight Analogs Project Standard Measure Testing Schedule

9.0 BED REST SUBJECT RECRUITMENT AND SCREENING

The NASA JSC Human Test Subject Facility (HTSF) advertises, recruits and pre-screens candidate subjects to meet unique campaign requirements.

Potential subjects both male and female age 24-55 are pre-screened via telephone interviews by HTSF nurses. Subjects who satisfy the basic inclusion criteria travel to Johnson Space Center for a NASA-modified Air Force Class III physical examination. Subjects are excluded from the study if they: a) are hypertensive, b) have electrocardiogram abnormalities, c) require medication that might interfere with the interpretation of the results (i.e. fluoride, steroids), d) have a recent sub-standard nutritional status, e) have metal implants which could interfere with MRI imaging, f) have a history of thyroid dysfunction, renal stones, mental illness, gastroesophageal reflux, cardiovascular disease, musculoskeletal or sensorimotor dysfunction, or have smoked within six months prior to the start of the study, g) have a personal or family history of thrombosis, h) have a body mass index (BMI) outside of 21-30, have abnormal blood or urine chemistries, or can not clear a criminal background check. Females must have regular menses, a negative pregnancy test, and not be using hormonal contraceptives. Screening for tuberculosis is also performed.

Blood chemistry testing includes fasting glucose, blood urea nitrogen (BUN), uric acid, creatinine, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline

phosphatase, lactate dehydrogenase, glutamyl transferase, sodium, potassium, chloride, phosphorous, calcium, magnesium,vitamin D, quantiferon gold, CO_2, total protein, cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, and high sensitivity C-reactive protein and an illicit and comprehensive urine drug screen.

A hematology profile is performed and includes white cell count and differential, red cell count, hemoglobin, hematocrit, ferritin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, red cell distribution width. Urinalysis includes pH, specific gravity, color, appearance, protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite, and leukocyte esterase.

Dual-energy x-ray absorptiometry (DXA), high-frequency QRS-EKG and 12-lead EKG are part of the screening. Subjects must meet minimum requirements to be placed in the study.

Following physical screening, subject candidates are tested and interviewed by a psychologist for assessment of their ability to complete all aspects of a study. Psychological screening methods utilize commercial, off-the-shelf psychological tests and International Classification of Diseases (ICD) criteria for psychiatric disorders, derived from current astronaut select-out and select-in procedures. Subjects are also assessed for roommate compatibility.

After medical and psychological clearance is received study specific screening tests are performed. If more that 30 days has elapsed since initial testing a hematology profile, fasting glucose, sodium, potassium, chloride, CO_2, BUN, creatinine, calcium, and electrocardiogram are repeated before study enrollment.

10.0 FLIGHT ANALOGS RESEARCH UNIT

- Bed rest research facility located at the Institute for Translational Sciences-Clinical Research Center (ITS –CRC) at the University of Texas Medical Branch
- Maximum capacity of 10 dedicated beds in semiprivate rooms available exclusively for NASA's use
- Common area for subjects to gather and meet with family and visitors
- On site space available to house Standard Measure and research equipment
- Core Laboratory facility with refrigerator, -8° freezer, and centrifuge
- Nursing staff
- Physician services-UTMB attending and medical monitoring (physicians with an out of state license are not eligible for privileges)
- UTMB staff psychiatrist
- Psychological Support Services
- Reconditioning post study
- Informatics support
    - intra and internet network access
    - telephone services
    - printers and copiers
    - radiology image transfer
- Metabolic kitchen

- The Metabolic Kitchen is staffed with a research dietician and experienced dietary personnel. They work with the NASA nutritional group to control the total energy and nutrient content of the research subject diets in a manner similar to the control of astronaut in-flight diets. This ensures the collection of comparable and appropriate data.

11.0 SUBJECT SERVICES

- Full time Activities Assistant employed to provide subjects with entertainment, holiday/birthday decorations, and arrange group activities.
- Individual subject laptop computer and TV on articulated arms to facilitate viewing.
- Internet access, cable TV, phone card, in room phone with local access.
- Access to movies, music, reading materials.
- Visitation privileges.
- Shower facility with 6 degrees head down tilt capability.

12.0 JSC LIFE SCIENCES RESEARCH LABORATORIES (LSRLs)

The Life Sciences Research Laboratories within the Human Adaptation and Countermeasures Division at NASA Johnson Space Center provide the expertise and hold the responsibility for collection and analysis of the FAP Standard Measures data. If a Standard Measure is the same or similar to a flight medical requirement the LSRLs also hold responsibility for collecting those data on crew members before and after spaceflight.

The LSRLs are paid by the Flight Analogs Project to collect these measures on FAP subjects and provide the FAP with the data, which can be shared with investigators.

13.0 FLIGHT ANALOGS DATA MANAGEMENT

- Archives raw data
- Life Sciences Data Warehouse serves as a repository for standard measure, subject specific, and investigator reduced data